# OOGENESIS AND RADIOSENSITIVITY IN COCHLIOMYIA HOMINIVORAX (DIPTERA: CALLIPHORIDAE)

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Ionizing radiation and some chemicals are known to render insects sterile. Sterility may be achieved in two ways, by a treatment that inhibits the formation or development of mature sperm or ova (fecundity), or by a treatment that does not deter the production of these cells *per se,* but induces dominant lethal changes in their hereditary material, rendering them incapable of sustaining embryonic growth or causing death in the post-embryonic stages (fertility). The end result—sterility —is the same, but the processes involved in producing it are quite different. Many studies have investigated the reproductive system of both males and females and their differences in radiosensitivity with respect to the induction of dominant lethals, but inhibition of the growth of the reproductive organs has not been so widely studied.

In many species, growth of the ovaries is difficult to measure, especially in organisms that produce only a few mature gametes at a time. In some insects, however, the females produce a large number of gametes synchronously (egg masses). When many cells are undergoing growth and maturation simultaneously in many ovarioles, the reproductive organs attain considerable size at sexual maturity and the effect of a treatment can be easily measured.

Some aspects of the development of the female reproductive system in the screwworm fly, *Cochliomyia hominivorax* (Cqrl.), have been reported, in connection with work on radiation-induced dominant lethal mutations in developing oocytes (LaChance and Leverich, 1962). The present studies are concerned with the inhibition of ovarian growth by radiation, and with the morphological and cytological changes that lead to sterility characterized by infecundity. Particular emphasis is given here to the early growth stages, which are especially radiosensitive, and to the comparative radiosensitivity of the stages in oogenesis.

## Materials and Methods

*Cochliomyia hominivorax* is an obligate parasite of warm-blooded animals. The biology and laboratory-rearing procedures for this insect have been discussed in several publications (see Bushland, 1960). The insects used in the experiments reported here were of the laboratory strain, reared on artificial medium. In this rearing technique, the larvae pass through the three instars in the artificial medium and pupate in sand. The length of the pupal period is approximately 7–7½ days at 80° F. The adults were reared at 80° F. and fed honey and water, a diet that supports life and ovarian growth very well. Laboratory-reared adults mate when approximately two days old. The females are gravid at 5–6 days of age,

and will produce an egg mass of more than 200 eggs when oviposition is induced by presenting the females with a small piece of lean meat and keeping them at a temperature of 90–96° F. for a few hours. A second egg mass may be produced 3–5 days later.

The radiation exposures were performed in air in a $Co^{60}$ radiation source (Jefferson, 1960) at a dose rate of 642–683 roentgens per minute. Calibration of the unit was accurate to ±6%.

In the studies of ovarian growth, both ovaries were dissected from individual females; 30 females were examined for each treatment group. The dimensions of each ovary were measured with an ocular micrometer in the eyepiece of a dissecting microscope (17 ×). One measurement of height and two of diameter (at right angles to each other) were made. Estimation of ovarian volume is somewhat difficult, since the organ does not resemble any geometric form precisely. The several formulae used to calculate the size of a rounded geometric object have certain values that are constant. However, since the measurements made in these experiments were not intended to establish an exact volume but to reduce the observations to a single number that could serve as a basis for comparison between groups, the volume of the ovaries was approximated by multiplying the three measurements and correcting for the magnification.

In the studies of ovarian growth in *Cochliomyia,* temperature was repeatedly observed to affect size: Females of equal age reared at 80° F. had larger ovaries than those reared at 73° F., and sexual maturity was reached in 5 days at the higher temperature whereas 7 days was required at the lower temperature. For this reason, rearing temperatures in these experiments were all closely controlled at 80° F.

For the cytological studies, whole mounts of ovarioles were made from females of various ages and stained on a microscope slide with the Feulgen procedure. In all studies, corroborative evidence was gained by examining freshly prepared mounts in insect saline with a Zeiss phase-contrast microscope at 1600 ×. The staining procedure causes considerable shrinkage of the ovarioles, so those examined in wet mounts were always somewhat larger than the Feulgen-stained preparations.

## Results and Discussion of Individual Experiments

### 1. *Growth of the ovaries in normal females*

Data on the measurements of the ovaries in normal females of various ages are summarized in Table I. Newly emerged females had immature ovaries with volumes ranging from 0.068 to 0.254 $mm^3$. The size of the ovary doubled between the first and second day of adult life and increased by more than five-fold between the second and third day of adult life. Growth then continued somewhat more slowly until the mature ovaries had attained a size of more than 7 $mm^3$. Thus, from emergence until sexual maturity, the size of the ovary increased approximately 60-fold.

The growth of the ovary is the manifestation of the growth processes occurring synchronously in the more than 100 ovarioles that comprise each ovary. A cytological study of the ovarioles was conducted to determine the sequence of events in normal oogenesis, so that a basis could be established for evaluating the effects of

TABLE I

*Growth of ovaries in normal Cochliomyia hominivorax. (Each number represents the mean from 30 females ± the standard error of the mean.)*

| Age of female (days ± 2 hours) | Mean volume of ovaries ± $s_{\bar{x}}$ (mm.$^3$) | |
|---|---|---|
| | Right | Left |
| 0–2 hours | 0.1423 ± 0.0225 | 0.1273 ± 0.0015 |
| 1 | 0.2022 ± 0.0095 | 0.2002 ± 0.0023 |
| 2 | 0.4094 ± 0.0470 | 0.3952 ± 0.0362 |
| 3 | 2.1064 ± 0.204 | 2.2446 ± 0.234 |
| 4 | 5.3841 ± 0.328 | 5.0761 ± 0.347 |
| 5 | 7.3885 ± 0.274 | 7.3921 ± 0.274 |

radiation or other damaging agents on the growth of the reproductive organs and on other factors affecting female sterility.

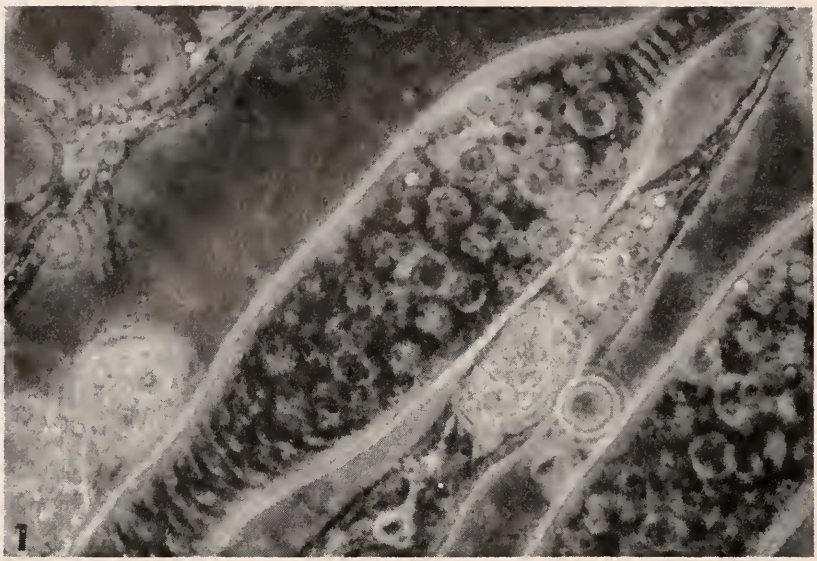
2. *Cytology of normal ovarioles*

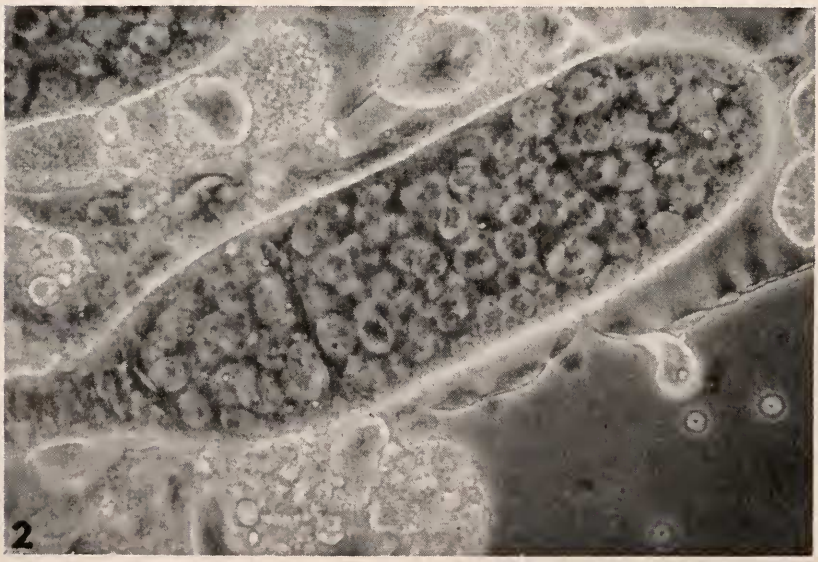
The ovary in *Cochliomyia* consists of 100–150 ovarioles, each of which is enclosed in an epithelial sheath and produces one mature egg to be deposited in each egg mass. Oocyte development is synchronous in all ovarioles. Scale drawings showing the normal developmental sequence in an ovariole are presented in Figures 8 and 9. The cytological studies showed the following pattern of oogenesis correlated with the age of the female.

*Pupae 4 and 5 days old.* The ovary is small and immature. Each ovariole is composed of a germarium filled with oogonial cells (Fig. 8A and Fig. 1). These cells are mitotically active, and nests of four or eight cells in division are occasionally seen in the anterior and middle portion of the germarium. There is no evidence of nurse cell formation. Examination of ovarioles from 4- and 5-day-old pupae, in Feulgen-stained preparations or in phase-contrast studies of fresh whole mounts in saline, did not show any differentiation of the germarial contents.

*Pupae 6 days old.* The ovariole is still composed of a single germarium, surrounded by an envelope of epithelial cells (Fig. 8B and Fig. 2). Mitotic divisions are now relatively rare. In some of the ovarioles the nuclei in the posterior portion of the germarium have enlarged very slightly, and the chromatin material has become slightly more diffuse. In some ovarioles a faint line of separation is seen between the anterior and posterior portion of the germarium (Fig. 2); this is the first indication of the first egg chamber being formed.

*Pupae 7 days old.* A cyst has formed in the posterior portion of each ovariole, indicating formation of the first egg chamber. In some ovarioles dissected from pupae almost ready to emerge, an indentation has formed that separates and "pinches off" the first egg chamber from the germarium (Figs. 3 and 8C). This newly formed chamber contains 16 cells, 15 of which are large and distinct nurse cells and the other, the smaller oocyte. The 15 nurse cells have not differentiated noticeably, except that the nuclei contain diffuse chromatin material. The germarium occasionally contains a smaller second cyst, in the posterior region, that is destined to become the second egg chamber (Fig. 3). This structure was visible in phase contrast; in Feulgen-stained preparations, changes in nuclear morphology were not observable in the remaining germarial contents.




FIGURES 1–2. Phase contrast, unfixed tissue in saline, 640 ×. Figure 1. Ovariole from untreated 5-day-old pupa. Figure 2. Ovariole from untreated 6-day-old pupa; note furrow forming in posterior region of germarium.

Presumably the 16 cells of an egg chamber derive from four consecutive divisions of an oogonial cell, maintain a positional relationship to one another, and are subsequently incorporated into an egg chamber or cyst, but in *Cochliomyia* there

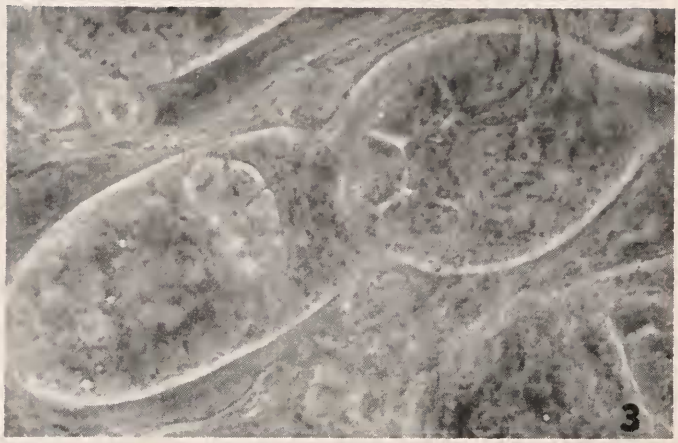
was no indication that these cells are enclosed in a cyst until the pupae are 6½–7 days old. Very likely the daughter cells are interconnected by fine protoplasmic strands, but these were not demonstrable with the techniques used in these studies. Hirschler (1955) has presented an excellent study of the early positional relationship of the cells that will subsequently develop into an egg-nurse cell complex in insects. He states (p. 78), "After a given number of divisions, the egg remains united to the conceivably greatest number of nurse-cells by means of cell bridges functioning as nutritive ducts. As far as nutrition is concerned, the egg lies in the best possible situation." The studies of King (1960) also indicate that these daughter cells remain connected by intercellular cytoplasmic bridges and are subsequently incorporated into an egg chamber. Fawcett *et al.* (1959) have studied the intercellular bridges that exist between daughter cells derived from an original parent cell in the spermatids of many species, and suggest that protoplasmic continuity between these cells is the basis for their synchronous development. It is quite possible that the synchronous development of ovarian nurse cells is also related to their intercellular connections.

*Adult females 0–4 hours old.* The 15 nurse cells in the first egg chamber are greatly enlarged (Fig. 8D). The chromosomes in the nurse cell nuclei are thickened and stain deeply with Feulgen stain (Fig. 4A and 4B). Formation of polytene chromosomes in ovarian nurse cells is quite common in Diptera (Stalker, 1954; Bier, 1960). In *Cochliomyia* the reduplicated chromonemal strands remain in close enough association to appear as greatly thickened chromosomes (Fig. 4A), but not so close as to give a very definite banded appearance as in some other species (Stalker, 1954; Bier, 1960).

*Adult females 4–24 hours old.* The major changes in the first egg chamber take place in the nurse cell nuclei, which continue to enlarge progressively, due to the replication of chromosomal material. This process results in the formation of polytene chromosomes. Chromonemal reproduction in the nurse cells is followed by a complete separation of the reproduced elements. The separation does not involve a regular dicentric anaphase, but merely a complete dissociation of the numerous strands or a general "falling apart," resulting in a mass of chromatin fibrils that completely fill the nuclear volume (Fig. 5). The entire process takes place within an intact nuclear membrane and may, according to the terminology of Lorz (1947) and others (see Painter and Reindorp, 1939; Painter, 1959), be termed endomitosis. This endomitotic process has been described in great detail by Painter and Reindorp (1939) and King (1960) for *Drosophila,* and by Bier (1960) for *Calliphora.* In *Cochliomyia* it is very similar, except that it takes place simultaneously in hundreds of ovarioles. Jacob and Sirlin (1959) have reported that in *Drosophila* the most posterior nurse cells undergo one more duplication than the more anterior cells, and become larger and more active. The posterior nurse cells in *Cochliomyia* are also distinctly larger (Fig. 5), and very likely undergo more replications of chromosomal material than do the anterior nurse cell nuclei.

In these studies, only rarely were the polytene chromosomes of the nurse cells observed to dissociate before the females were 8 hours old. In ovarioles from females 8–16 hours old, the thickened polytene chromosomes had dissociated into Feulgen-positive chromatin threads in approximately half of the nurse cells.

*Adult females 24 hours old.* The endomitotic process is completed during the first day of adult life. After 24 hours all the nurse cell nuclei are filled with loosely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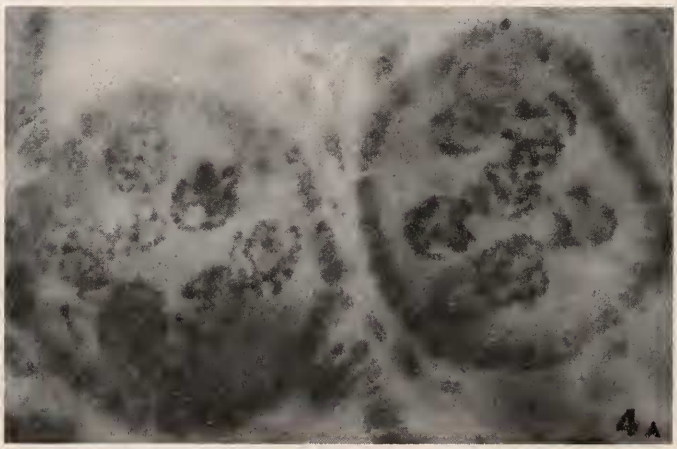
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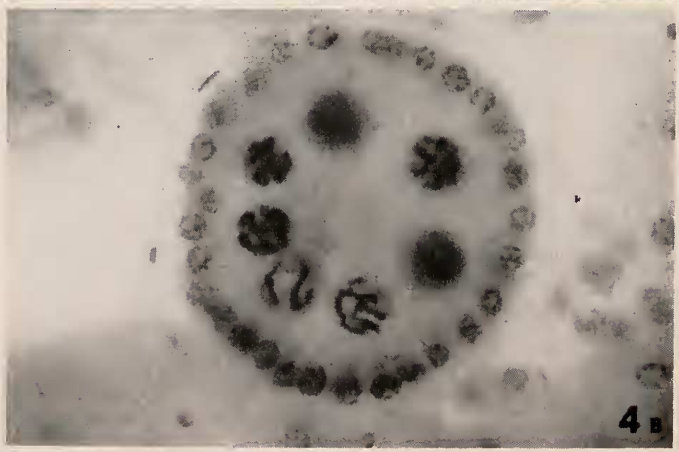
4B

associated Feulgen-positive chromatin threads (Fig. 5). The nurse cells have now enlarged to their greatest size. The ovariole consists of an enlarged first egg chamber, the second chamber is beginning to form in some ovarioles, and the germarium is filled with oogonial cells (Fig. 9A). In contrast, the oocyte nucleus in newly emerged females is smaller and stains very lightly with Feulgen stain throughout the first day. In these studies the nucleolus did not stain, but it was clearly visible in phase-contrast examination.

*Adult females 48 hours old.* The first egg chamber has enlarged considerably, so that the nurse cells are not so closely packed, but more dispersed in the chamber. The oocyte nucleus is very small and spherical, and is located in the posterior region of the egg chamber. The second egg chamber has now clearly formed in all ovarioles. The nurse cells in the first egg chamber are extruding a fine granular material which stains faintly with Feulgen; this material is beginning to fill the egg chamber.

*Adult females 3 days old.* Between the second and third day after emergence, the first egg chamber in each ovariole more than doubles in size (see Table I). The nurse cells are localized at the anterior end of the chamber (Fig. 9B and C). The oocyte has grown noticeably and is beginning to elongate; the oocyte nucleus is located in the ooplasm very near the nurse cells and is in prophase I of the first meiotic division (LaChance and Leverich, 1962). The second egg chamber has also enlarged and contains nurse cells undergoing endomitotic replications. Some of the nurse cells in the second egg chamber have completed the process of endomitotic growth and dissociated chromosomal material is beginning to fill the nucleus.

*Adult females 4 days old.* The mature ovum is now almost fully formed. The nurse cells, when present, stain much more deeply and appear to darken and disintegrate. Most nurse cells in the upper portion of the egg follicle have disappeared (Fig. 9D). The disappearance of the nurse cells from the first egg chamber is correlated with the passage of the oocyte nucleus from prophase I to metaphase I of the first meiotic division (LaChance and Leverich, 1962).

*Adult females 5 days old.* Each ovariole contains a mature ovum ready for oviposition (Fig. 7a). The nurse cells have completely disappeared and the oocyte nucleus is now in anaphase I of the first meiotic division (LaChance and Leverich, 1962). All nurse cells in the second egg chamber have completed endomitotic growth and contain nuclei filled with fine Feulgen-positive threads existing in great multiplicity. A very small third egg chamber has just formed from the germarium.

Thus, at the time when the first egg mass is deposited, each ovariole contains a well developed second egg chamber, which will then repeat the process described above and form a mature ovum to be deposited in the second egg mass. At 5 days of age, growth in the ovarioles is arrested until deposition of the first egg mass; cytological preparations of ovarioles from females 7–9 days of age that have not oviposited closely resemble those of females 5 days of age.

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FIGURE 3. Phase contrast, unfixed tissue in saline, 640 ×. Ovariole from untreated pupa 7–7½ days old; first egg chamber well formed (upper right), germarium containing a second cyst (lower left).

FIGURE 4. Feulgen-stained whole mounts, 800 ×. Egg chambers in ovarioles from untreated adults 0–4 hours old. (A) Two adjoining egg chambers, showing polytene nurse cell chromosomes (lower focal level). (B) One egg chamber, showing polytene nurse cell chromosomes (upper focal level).

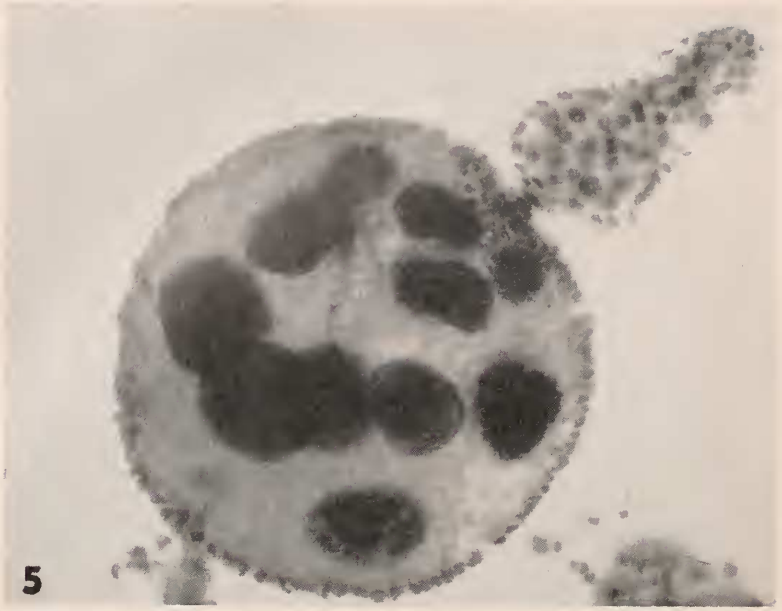


FIGURE 5. Feulgen-stained whole mount, 512×. Ovariole from untreated 28-hour-old adult; note 15 nurse cell nuclei containing dissociated chromatin fibrils, oocyte nucleus very faintly stained (lower left of first egg chamber).

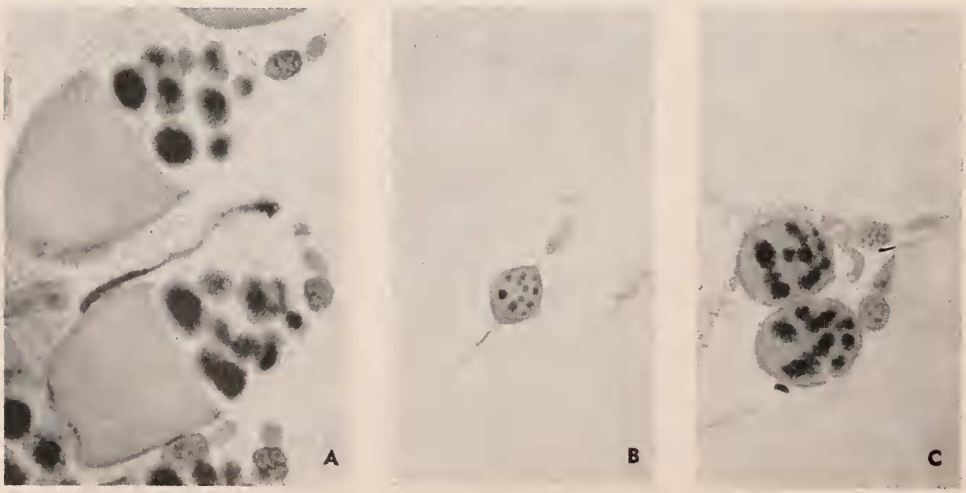


FIGURE 6. Feulgen-stained whole mounts, 80×. Ovarioles from 2½-day-old adults. (A) Control; shows large first egg chamber with 15 enlarged nurse cells, second egg chamber, and germarium. (B) Treated with 2000 r as 5-day-old pupa; note absence of second egg chamber. (C) Treated with 2000 r as adult 0-4 hours old.

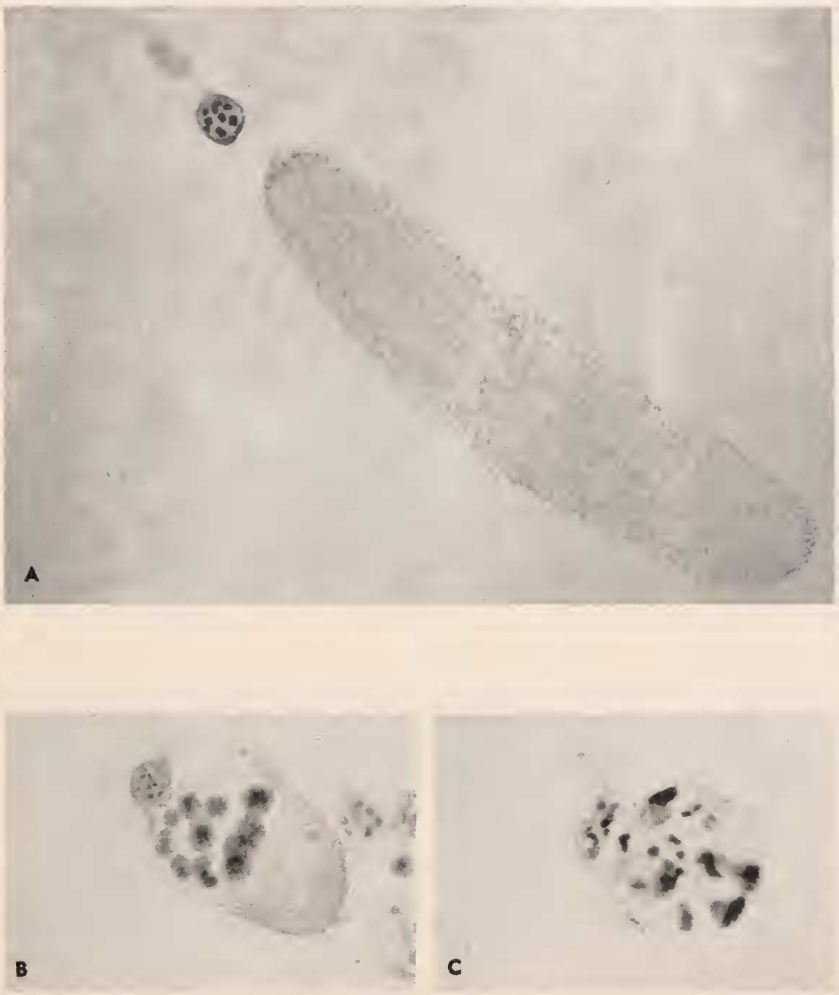


FIGURE 7. Feulgen-stained whole mounts, 80×. Ovarioles from 4½-day-old adults. (A) Control. (B) Treated with 4000 r as 5-day-old pupa; note typical undeveloped first and second egg chambers and atrophied germarium. (C) Treated with 4000 r as adult 0–4 hours old; note typical malformed egg chambers and germarium.

### 3. *Effects of irradiation on ovarian growth*

With the data obtained on ovarian measurements and the cytological studies of ovarioles from normal females of various ages, it was possible to study the effects of gamma radiation treatments, and to determine which ages presented the reproductive system in stages of highest radiosensitivity or resistance. By observing the inhibition of ovarian growth induced in females of various ages by a given dose of radiation, it was possible to compare the sensitivity of oogonial cells, of egg chambers in which the nurse cells were undergoing endomitotic growth, and of

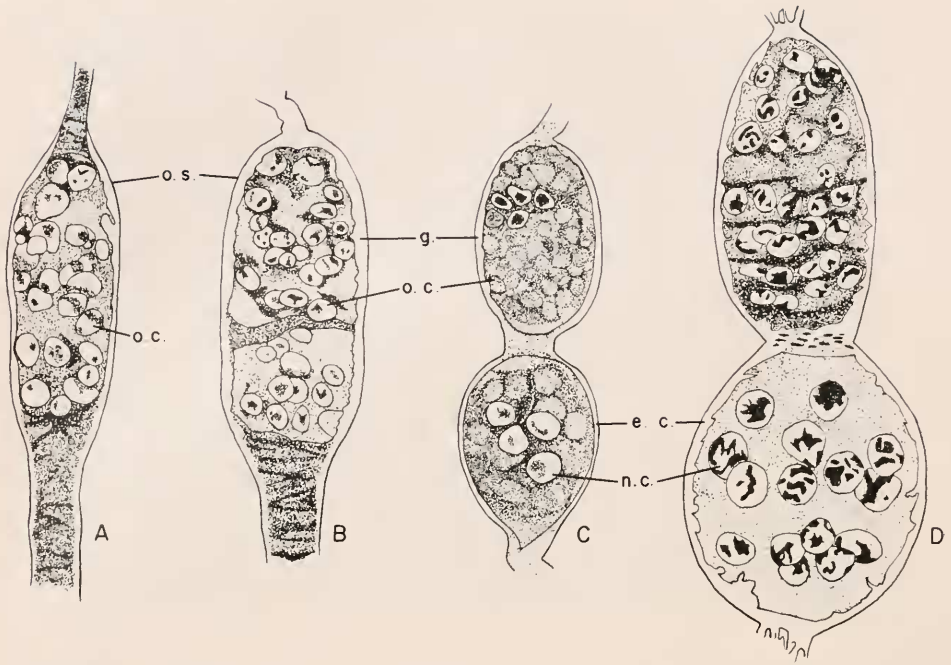


FIGURE 8. Normal ovarian development in *Cochliomyia hominivorax*. Each figure represents a single ovariole; drawings prepared from unfixed whole mounts examined in phase contrast (enlarged 400×). (A) From pupa 4½–5 days old. (B) From pupa 6 days old. (C) From pupa 7½–8 days old. (D) From adult 0–4 hours old. (o.s. = ovariole sheath; g. = germarium; e.c. = first egg chamber; o.c. = oogonial cells; n.c. = nurse cells.)

older egg chambers in which endomitosis was completed but vitellogenesis was in progress. The results of these comparisons are presented in Table II.

The early experiments of Bushland and Hopkins (1953) showed that larvae and young pupae were severely injured by irradiation and emergence was reduced. However, 6-day-old pupae could be sterilized by a dose of 5000 r. When pupae were treated at this level, most of the adult females did not produce eggs and the few that did produced a very few eggs, which did not hatch; the adult males produced motile sperm that contained dominant lethals. In further work on 6-day-old pupae (Baumhover *et al.*, 1955), it was found that a dose of 7500 r was required to prevent egg production completely. The studies in Table II show that the process of ovarian growth can be inhibited at other times in the life cycle with smaller doses of radiation. Thus, although between 4000 and 5000 r were required to inhibit growth of the ovaries when 5-day-old pupae were irradiated, newly emerged females were much more radiosensitive: a dose of 2000 r reduced ovarian growth by half, and almost complete inhibition of ovarian growth was obtained with 4000 r. However, when two-day-old females were given even higher doses (up to 8000 r), ovarian growth and egg production were not greatly affected. It should be noted that, although irradiation of older females does not greatly inhibit

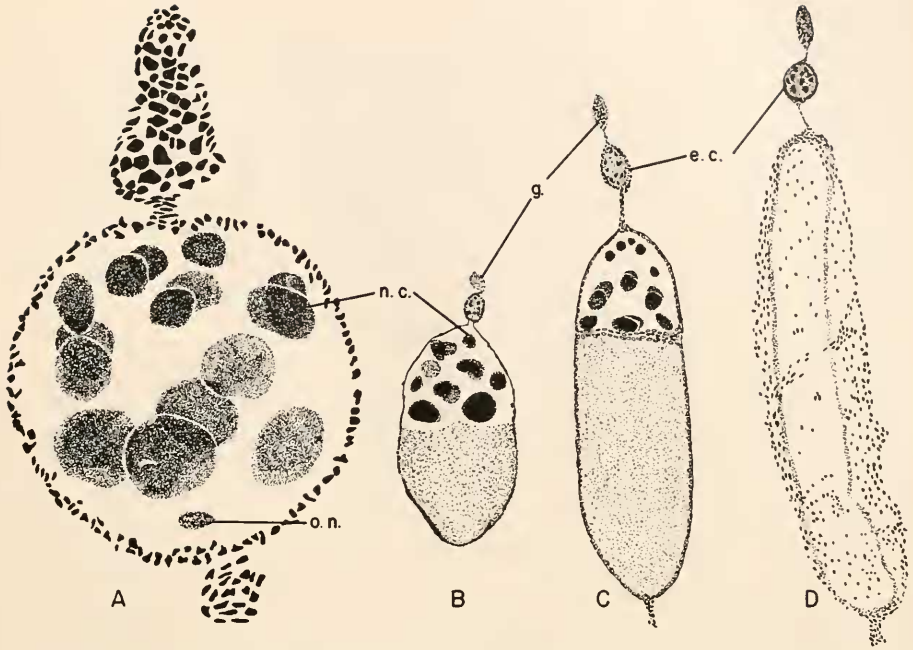


FIGURE 9. Normal ovarian development in *Cochliomyia hominivorax*. Each figure represents a single ovariole; drawings prepared from stained material. (A) From adult 28 hours old (384×). (B) From adult 2½ days old (60×). (C) From adult 3½ days old (60×). (D) From adult 4 days old (60×). (n.c. = nurse cells; o.n. = oocyte nucleus; e.c. = second egg chamber; g = germarium.)

ovarian growth, sterility can still be achieved by inducing dominant lethal changes in the formed or forming oocytes so that, even though eggs are produced, embryos will not develop (LaChance and Leverich, 1962).

TABLE II

*Effects of gamma radiation on ovarian growth in Cochliomyia hominivorax. (Each number represents the mean from 30 5-day-old females ± the standard error of the mean.)*

| Radiation dose (r) | Mean volume of ovaries ± $s_{\bar{x}}$ (mm.³) from females treated as: | | | | | |
|---|---|---|---|---|---|---|
| | 5-day-old pupae | | Adults 0–4 hours old | | Adults 48–52 hours old | |
| | Right | Left | Right | Left | Right | Left |
| Control | 8.1410 ±0.277 | 8.3247 ±0.375 | 8.3400 ±0.350 | 8.3529 ±0.381 | 8.3400 ±0.350 | 8.3529 ±0.381 |
| 2000 | 8.1102 ±0.264 | 8.1696 ±0.276 | 4.4966 ±0.403 | 4.3916 ±0.426 | 7.4108 ±0.356 | 7.3072 ±0.344 |
| 4000 | 3.6555 ±0.278 | 3.6035 ±0.251 | 1.7251 ±0.142 | 1.6723 ±0.104 | 6.4428 ±0.390 | 6.7258 ±0.421 |
| 5000 | 0.9583 ±0.120 | 0.9077 ±0.112 | 1.2828 ±0.074 | 1.2200 ±0.078 | 7.1426 ±0.332 | 7.3094 ±0.348 |
| 6000 | 0.4327 ±0.033 | 0.4511 ±0.040 | 0.8968 ±0.069 | 0.8867 ±0.068 | 6.5205 ±0.375 | 6.4219 ±0.424 |
| 8000 | 0.2503 ±0.018 | 0.2646 ±0.018 | 1.0820 ±0.275* | 1.1390 ±0.293* | 7.0346 ±0.419 | 6.9750 ±0.426 |

* Mean from 18 females only.

4. *Cytology of the irradiated ovary*

To investigate the cytopathological changes related to the inhibition of ovarian growth after irradiation, a further series of experiments was conducted. Females were irradiated with 2000 or 4000 r, either as 5-day-old pupae or as newly emerged adults 0–4 hours old. The ovaries were dissected from the females at $2\frac{1}{2}$, $4\frac{1}{2}$, and $5\frac{1}{2}$ days after emergence and the ovarioles teased apart, fixed, and stained. The slides were made permanent and were later examined microscopically for the degree of development, presence or absence of nurse cells, presence or absence of second egg chambers, and staining differences in the nuclear components, as compared with a suitable set of controls. The slides were coded before examination to prevent bias in recorded observation. The results are presented in Table III.

Occasionally in the dissecting and staining procedure, some portion of the ovariole was torn or lost. This condition occurred most frequently in the area between the first and second egg chambers, which are connected by a very thin sheath. Therefore, at times only the first egg chamber could be observed for cytological changes after a treatment, and the condition of the rest of the ovariole remained unknown. For this reason, there are more first egg chambers recorded in Table III than second egg chambers or germaria. In treatment group A, for example, 150 ovarioles were examined, but of these only 114 included the second egg chamber, and 103 contained the complete ovariole including the germarium. In no instance was any component scored as absent if any possibility existed that it had been lost in dissection; when second egg chambers are recorded as absent, both the first egg chamber and the germarium were present within the ovariole sheath, and the second egg chamber had clearly failed to develop (Fig. 6B).

Altogether, 1406 ovarioles were examined. The amount of information originally collected has been summarized for presentation in Table III. In order to organize the data, it was necessary to separate the appearance of ovarioles and their contents into categories as follows:

*Reduced normal* indicates that the entire structure, including nurse cells, appeared normal in shape and degree of staining, but was distinctly smaller in size than controls of similar age. This term for the first egg chamber indicates that the nurse cells had begun to migrate toward one end of the follicle and that 15 nurse cells were present in nearly all instances. For the second egg chamber, all the structures were present but reduced in size. *Undeveloped* refers to the first egg chambers and indicates that the chambers were very small but usually contained 15 nurse cells. Normal development had been retarded so that, in comparison with controls of similar age, the ovarioles appeared much younger as well as smaller. The nurse cells in the chamber were scattered throughout the entire structure rather than tending to gather at the polar end (Fig. 6C). *Malformed* also refers to first egg chambers and is used to describe ovarioles from the older groups ($4\frac{1}{2}$ and $5\frac{1}{2}$ days old when dissected) that were so badly stunted in size and development that they could not even be considered "undeveloped" (Fig. 7C). Many egg chambers were misshapen and contained pycnotic or degenerate nurse cells; the chambers were extremely small for their age and showed no signs of development.

*Absent* in reference to the second egg chamber indicates a complete lack of a recognizable object between the first egg chamber and the germarium, and a vacant space in the ovariole sheath (Fig. 6B). *Atrophied* means that a second egg

TABLE III

*Cytopathology of the irradiated ovary in Cochliomyia hominivorax. Females treated with gamma radiation and the ovarioles dissected for study at indicated days post-treatment. See text for definition of column headings*

| Treatment | | | | Number observed | First egg chamber | | | | | | Second egg chamber | | | | | | Germarium | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Group | Dose (r) | Age treated | Age dissected (days) | | Reduced normal | | Undeveloped | | Malformed | | Reduced normal | | Absent | | Atrophied | | Reduced normal | | Degenerate | |
| | | | | | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % | No. | % |
| A | 2000 | 5-day pupae | 2½ | 150 | | | 150 | 100.0 | | | 78 | 68.4 | 36 | 31.6 | | | 103 | 100.0 | | |
| B | 2000 | 5-day pupae | 4½ | 120 | 105 | 87.5 | 15 | 12.5 | | | 7 | 87.5 | 1 | 12.5 | | | 6 | 100.0 | | |
| | | | | Total 270 | 105 | 38.9 | 165 | 61.1 | | | 85 | 69.6 | 37 | 30.3 | | | 109 | 100.0 | | |
| C | 2000 | 0–4-hour adults | 2½ | 208 | 101 | 48.6 | 107 | 51.4 | | | 134 | 85.9 | 22 | 14.1 | | | 144 | 99.3 | 1 | 0.7 |
| D | 2000 | 0–4-hour adults | 4½ | 79 | 25 | 31.6 | 9 | 11.4 | 45 | 57.0 | 33 | 89.2 | 4 | 10.8 | | | 25 | 78.1 | 7 | 21.9 |
| E | 2000 | 0–4-hour adults | 5½ | 76 | 1 | 1.3 | | | 75 | 98.7 | 36 | 83.7 | 1 | 2.3 | 6 | 14.0 | 29 | 78.4 | 8 | 21.6 |
| | | | | Total 363 | 127 | 35.0 | 116 | 32.0 | 120 | 33.1 | 203 | 86.0 | 27 | 11.4 | 6 | 2.5 | 198 | 92.5 | 16 | 7.5 |
| F | 4000 | 5-day pupae | 2½ | 102 | | | 102 | 100.0 | | | 28 | 50.9 | 27 | 49.1 | | | 45 | 100.0 | | |
| G | 4000 | 5-day pupae | 4½ | 66 | | | 66 | 100.0 | | | 3 | 14.3 | 18 | 85.7 | | | 9 | 60.0 | 6 | 40.0 |
| | | | | Total 168 | | | 168 | 100.0 | | | 31 | 40.8 | 45 | 59.2 | | | 54 | 90.0 | 6 | 10.0 |
| H | 4000 | 0–4-hour adults | 2½ | 172 | | | 172 | 100.0 | | | 65 | 59.1 | 45 | 40.9 | | | 70 | 68.0 | 33 | 32.0 |
| I | 4000 | 0–4-hour adults | 4½ | 146 | | | | | 146 | 100.0 | 70 | 85.4 | 7 | 8.5 | 5 | 6.1 | 58 | 87.9 | 8 | 12.1 |
| J | 4000 | 0–4-hour adults | 5½ | 60 | 2 | 3.3 | 56 | 93.3 | 2 | 3.3 | 1 | 5.3 | 18 | 94.7 | | | 8 | 42.1 | 11 | 57.9 |
| | | | | Total 378 | 2 | 0.5 | 228 | 60.3 | 148 | 39.1 | 136 | 64.5 | 70 | 33.2 | 5 | 2.4 | 136 | 72.3 | 52 | 27.7 |

chamber was present but small and misshapen, and frequently with no nurse cells or very poorly defined ones.

A *reduced normal* condition for the germarium means the same as for the first and second egg chambers: The structure appeared normal but was much smaller in size than controls of the same age. A *degenerate* germarium indicates that there was evidence of the presence of a germarium but that it was so shrunken in appearance that it could not be considered merely reduced in size (Fig. 7B).

Controls for these experiments were untreated females dissected at $2\frac{1}{2}$, $4\frac{1}{2}$, and $5\frac{1}{2}$ days after emergence. The appearance of normal ovarioles at various ages has been described in section 2, and photographs are given in Figures 6A and 7A.

Some cytological observations from these experiments could not be readily categorized for presentation in Table III, and are discussed in the following paragraphs. These comments are intended to supplement the data in Table III. The data in the table were based on comparisons of ovarioles from the control groups with those from treatment groups. *No* abnormalities were found in the controls, but in none of the treated females was completely normal development of an egg chamber ever observed, even at low, non-sterilizing doses of radiation. All ovarioles from treated females were distinctly retarded in growth even if nothing else appeared abnormal.

*Five-day-old pupae treated with 2000 r (treatment groups A and B).* Of 150 ovarioles dissected from females $2\frac{1}{2}$ days after emergence (group A), all could be classified as undeveloped, and 10 of these were very small with tiny nurse cells. This pattern was interpreted as retardation of growth rather than cessation, for when ovarioles from similarly treated females $4\frac{1}{2}$ days old were examined (group B), 87.5% had developed to a stage that would normally be found in control females two or three days old—that is, the nurse cells were very small but still evident and occupying half the volume of the first egg chamber. Of the 270 first egg chambers examined in the two groups, all had 15 nurse cells that were reduced in size. Undeveloped nurse cells could result in a much slower rate of vitellogenesis and thus account for the small or undeveloped egg chambers. The observations on second egg chambers indicated that these also formed at a much later time than in normal ovarioles. When ovarioles from $2\frac{1}{2}$-day-old females were examined, 31.6% had failed to develop second egg chambers; of the ovarioles from $4\frac{1}{2}$-day-old females, 87.5% had a second egg chamber but it was small for the age group. Apparently the process of ovogenesis in the contents of the ovarioles was retarded at least two days by this treatment. The most obvious trend was a slowing down of all processes involved in ovogenesis, but not a complete cessation of development.

*Adults 0–4 hours old treated with 2000 r (treatment groups C, D, and E).* Of the 208 ovarioles dissected at $2\frac{1}{2}$ days (group C), all had 15 nurse cells that were normal in appearance but reduced in size. In the 79 ovarioles dissected from $4\frac{1}{2}$-day-old females (group D), 45 had malformed ova; 34 had egg chambers that were reduced in size or undeveloped, and of these 26 had 15 nurse cells that were reduced in size, 4 had less than 15 nurse cells, and 4 had pycnotic or degenerate nurse cells numbering 14 or less. In the 76 ovarioles dissected from females at $5\frac{1}{2}$ days (group E), 98.7% of the first egg chambers examined were malformed, and most of these lacked nurse cells or contained shrunken, degenerate nurse cells.

In these three treatment groups, 236 ovarioles were complete enough to permit observation of second egg chambers, but in only 203 of these were the second egg chambers developed. In group D, of 37 complete ovarioles examined, 4 were lacking a second egg chamber, 25 had formed a second chamber in which there were 15 small nurse cells, 7 had formed a chamber with definitely abnormal nurse cells, and one had a chamber with less than 15 nurse cells. Most germaria observed in the complete ovarioles were normal in appearance but reduced in size when compared with controls of similar age; 16 were completely degenerate and misshapen.

In general, the damage to the oocytes was greater after treatment of females 0–4 hours old with 2000 r than after a like treatment of 5-day-old pupae (see Table II). Instead of merely slowing down the process of ovogenesis and allowing the delayed production of some normal eggs, treatment of newly emerged females seemed to result in a preponderance of malformed eggs. Although the second egg chambers were usually developed to some extent (86%), their growth was reduced considerably and was occasionally followed by atrophy. In addition, degeneration of germaria was observed; this condition did not occur after treatment of 5-day-old pupae.

*Five-day-old pupae treated with 4000 r (treatment groups F and G).* The first egg chambers that developed following treatment with 4000 r grew only very slightly and then remained almost stationary in size. Of the 168 first egg chambers examined, none had progressed past the "undeveloped" stage. Of 66 first egg chambers dissected at 4½ days, 47 contained 15 nurse cells of reduced size, 17 contained from 2 to 14 small nurse cells of nearly normal appearance, and two contained pycnotic or degenerate nurse cells.

In these two treatment groups, 76 complete ovarioles were observed, but in only 31 of these were second egg chambers developed. Of the 55 complete ovarioles dissected at 2½ days, second egg chambers were absent in 27 ovarioles; second chambers had formed in 28 ovarioles but were extremely reduced in size, and in only 8 of these was it possible to observe 15 nurse cells. Of the 21 complete ovarioles dissected at 4½ days, 18 did not form second egg chambers, and of the remaining three that did, 15 nurse cells were observed in only one and these were reduced in size.

The general trend of ovarian development after treatment of 5-day-old pupae with 4000 r was a retardation of growth in the first egg chamber. There were no instances of the first egg chamber progressing to the point of formation of an ovum without nurse cells; growth was apparently arrested at an early stage of development. Second egg chambers had failed to develop in 60% of the complete ovarioles studied, and both reduction in growth rate and atrophy were observed in the germaria.

*Adults 0–4 hours old treated with 4000 r (treatment groups H, I, and J).* Of the 378 first egg chambers observed, 228 were undeveloped and 148 had developed into malformed and abnormal oocytes (Fig. 7C). Of 172 first egg chambers from females dissected at 2½ days (group H), all appeared undeveloped, with 15 nurse cells of reduced size. Of 146 first egg chambers from 4½-day-old females (group I), all were both undeveloped and malformed; 58 of the follicles still had 15 very small nurse cells, but in the remaining 88 the nurse cells had begun to degenerate and

were difficult to count precisely (Fig. 7C). Of the 60 ovarioles from 5½-day-old females (group J), only two could be classified as normal in appearance but distinctly retarded in growth; 56 were still undeveloped, and two malformed. Of the first egg chambers from these 60 ovarioles, 35 contained approximately 15 nurse cells of reduced size and 25 contained pycnotic or degenerate nurse cells.

Of 211 complete ovarioles from these three groups, the second egg chamber had failed to develop in 70, in 5 it was atrophied, and in 136 it was very much reduced in size. There was a relatively high incidence (28%) of germaria being found in a degenerate state or absent altogether; in all other instances in which the germarium was observed, its size was reduced.

Previous work has shown that a treatment of 4000 r given to females 0–4 hours old results in complete sterility (LaChance and Leverich, 1962), whereas a similar treatment of 5-day-old pupae results in considerable damage to the reproductive tissues but does not fully sterilize the females (Bushland and Hopkins, 1953).

## General Discussion

It is tempting to compare the mode of action of the various agents that are known to cause sterility. Since *Cochliomyia hominivorax* is currently being tested with a number of chemosterilants, it is hoped that the present observations on the pattern of radiation-induced sterility in the female of this species will serve as a basis for future comparisons against the effects of chemical sterilization.

The present studies demonstrate that the effect of irradiation on the reproductive capability of female *Cochliomyia* is largely dependent on the stage of development of the ovarioles at the time the radiation treatment is administered. The time during which the egg chambers contain nurse cells undergoing endomitotic replications of chromosomal material (adults 0–4 hours old) was the most radiosensitive stage encountered in these studies. Irradiation during this period is much more likely to be followed by infecundity than when an equivalent dose of radiation is delivered before endomitosis begins (5-day-old pupae) or after it has been completed (24-hour-old adults) (see Table II).

The present studies corroborate the observations of Grosch and Sullivan (1954) that the period of endomitosis is especially vulnerable to damage by irradiation and that, following treatment at this stage, growth is hampered. There are several possible reasons why a group of nurse cells would present an especially vulnerable target during the endomitotic process. Although it is true that the polyploid nurse cells in very young females present a multiplicity of chromosomal targets as compared with the diploid oogonial cells in 5-day-old pupae, it is not likely that greater radiosensitivity is associated merely with the greater content of DNA in the nurse cells. The nurse cells in females 24 hours old certainly contain as much, or more, DNA as those of younger females, yet 24-hour-old females are so resistant to radiation treatments that even doses of 8000 r do not seriously hamper egg production. Rather, we favor the idea that the failure of the treated females to produce mature ova reflects an inability of the nurse cells to support normal vitellogenesis. If a radiation treatment is given before the endomitotic replication of nurse cell chromosomal material is completed, the process is likely to be arrested, and the result is the formation of nurse cells without the elevated complement of chromosomal material

in the nuclei. King and Sang (1959) have suggested that vitellogenesis cannot proceed to completion without this elevated chromosomal complement. One of the most common features in the present cytological studies of irradiated ovarioles was nurse cells that were fairly normal in appearance but very much reduced in size.

The formation of immature egg chambers with abnormally small nurse cells could lead to such changes in the process of oogenesis as a slowing down of vitellogenesis or, often, a general cessation of growth at the point at which vitellogenesis would normally be most active and contribute most to further increases in the size of the oocyte. Occasionally the nurse cells degenerated before growth of the oocyte was complete, which resulted in the production of very few eggs or malformed eggs that did not hatch.

On the other hand, there remains the possibility that the oocyte nucleus changes in radiosensitivity during the various stages of oogenesis. Damage to the oocyte nucleus could conceivably affect the process of vitellogenesis by removing in some manner the stimulus for the nurse cells to produce yolk. There is some evidence that this factor operates in *Drosophila:* In studies of ovarian tumors in this species, King *et al.* (1961) observed that nurse cells will form without the presence of an oocyte, but that yolk is not produced without an oocyte nucleus in the chamber.

Ovarian tumors similar to those observed by King (1957) were not found in the present experiments with female *Cochliomyia.* This failure to observe ovarian tumors can be attributed to the fact that such tumors are relatively rare (in the *Drosophila* experiments only 13 tumorous egg chambers were observed per 10,000 cells examined when females were treated with 4000 r). More important, the two species differ in the pattern of egg production. Ovarian tumors are of clonal origin and arise from oogonial cells that mutate before the formation of a 16-cell cyst. Since the egg chambers examined in the present cytological studies were derived from cells that had probably already undergone the required number of somatic divisions to form a cyst, it was not expected that this type of tumor would be found.

The irradiation of developmental stages in *Drosophila* has resulted in a reduced number of ovarioles, but in *Habrobracon juglandis* (Ashmead) (= *Bracon hebetor* Say), the normal number of ovarioles always forms after treatment of various growth stages (larvae and pupae) with a number of different radiation doses (Erdman, 1961). In this respect, *Cochliomyia* resembles *Habrobracon:* The present studies clearly indicate that the reduction of growth in the ovary is not due to a reduction in the number of ovarioles comprising the ovary, but rather to morphological changes in the ovarioles, which persist in normal numbers.

Failure of mature ova to form is most often associated with a failure of the 15 nurse cells to function normally. However, clear instances in which egg chambers had formed with less than 15 nurse cells were found after irradiation of both 5-day-old pupae and adults 0–4 hours old; in all such instances growth of the egg chamber was seriously hampered. In contrast, King *et al.* (1961) have shown that in *Drosophila,* vitellogenesis in oocytes can proceed with as few as 10 or as many as 30 nurse cell nuclei. It is presumed, however, that for a reduced number of nurse cells to support vitellogenesis, the nurse cells must contain at least the normal polyploid number of chromosomes.

## Summary

1. In normal *Cochliomyia hominivorax* females, gross ovarian growth, correlated with the age of the adult from emergence to sexual maturity, was measured. Studies showed that the size of the ovary doubles between the first and second day of adult life, increases more than 5-fold between the second and third day, and exhibits a total increase of approximately 60-fold from emergence to sexual maturity.

2. A cytological study of the ovarioles was conducted to determine the sequence of events in normal oogenesis. The cytology of the reproductive system, from 5-day-old pupae to sexually mature females, is described.

3. The effects of gamma radiation on gross ovarian growth indicated that newly emerged females are more radiosensitive than 5-day-old pupae, and that irradiation of 2-day-old females has little effect on subsequent ovarian growth.

4. The cytopathology of the irradiated ovary was studied after similar doses of radiation were delivered to various developmental stages. The general sequence of events after treatment was as follows: After low doses, growth is slower than normal but not completely arrested. If treatment is given to 5-day-old pupae, grossly malformed oocytes are not often encountered, but second egg chambers frequently do not form. When females 0–4 hours old are irradiated, growth in the first egg chamber is delayed considerably, and is often followed by complete degeneration of the first egg follicle or the formation of grossly malformed oocytes. In these studies, the most radiosensitive stage encountered was that period during which the egg chambers contain nurse cells undergoing endomitotic replications of chromosomal material.

## LITERATURE CITED

Baumhover, A. H., A. J. Graham, B. A. Bitter, D. E. Hopkins, W. D. New, F. H. Dudley and R. C. Bushland, 1955. Screw-worm control through release of sterilized flies. *J. Econ. Ent.*, **48**: 462–466.

Bier, K., 1960. Der Karyotyp von *Calliphora erythrocephala* Meigen unter besonderer Berücksichtigung der Nahrzellkernchromosomen im gebundelten und gepaarten Zustand. *Chromosoma,* **11**: 335–364.

Bushland, R. C., 1960. Screw-worm research and eradication. *Advances in Vet. Sci.,* **6**: 1–18.

Bushland, R. C., and D. E. Hopkins, 1953. Sterilization of screw-worm flies with X-rays and gamma rays. *J. Econ. Ent.,* **46**: 648–656.

Erdman, H. E., 1961. Analyses of the differential radiosensitivity of developing reproductive tissues in *Habrobracon juglandis* (Ashmead) to ionizing radiations. *Int. J. Rad. Biol.,* 3: 183–204.

Fawcett, D. W., S. Ito and D. B. Slautterback, 1959. The occurrence of intercellular bridges in groups of cells exhibiting synchronous differentiation. *J. Biophys & Biochem. Cytol.,* **5**: 453–460.

Grosch, D. S., and R. L. Sullivan, 1954. The quantitative aspects of permanent and temporary sterility induced in female *Habrobracon* by x-rays and beta radiation. *Rad. Research,* 1: 294–320.

Hirschler, J., 1955. On the cooperation of fusomes in the development of egg-nurse cell complexes in the animal ovary. *La Cellule,* **57**: 67–87.

Jacob, J., and J. L. Sirlin, 1959. Cell function in the ovary of *Drosophila melanogaster.* I. DNA classes in the nurse cell as determined by autoradiography. *Chromosoma,* **10**: 210–228.

Jefferson, M. E., 1960. Irradiated males eliminate screw-worm flies. *Nucleonics,* **18**: 74–76.

King, R. C., 1957. Oogenesis in adult *Drosophila melanogaster.* III. Radiation-induced ovarian tumors. *Growth,* **21**: 129–135.

KING, R. C., 1960. Oogenesis in adult *Drosophila melanogaster*. IX. Studies on the cytochemistry and ultrastructure of developing oocytes. *Growth*, **24**: 265–323.

KING, R. C., AND J. H. SANG, 1959. Oogenesis in adult *Drosophila melanogaster*. VIII. The role of folic acid in oogenesis. *Growth*, **23**: 37–53.

KING, R. C., E. A. KOCH AND G. A. CASSENS, 1961. The effect of temperature upon ovarian tumors of the Fes mutant of *Drosophila melanogaster*. *Growth*, **25**: 45–65.

LACHANCE, L. E., AND A. P. LEVERICH, 1962. Radiosensitivity of developing reproductive cells in female *Cochliomyia hominivorax*. *Genetics*, **47**: 721–735.

LORZ, A. P., 1947. Supernumerary chromonemal reproductions: Polytene chromosomes, endomitosis, multiple chromosome complexes, polysomaty. *Botan. Reviews*, **13**: 597–624.

PAINTER, T. S., 1959. Some values of endomitosis. *In:* Biological Contributions. M. R. Wheeler (ed.), Univ. of Texas Publ. #5914, University Press, Austin, pp. 235–240.

PAINTER, T. S., AND E. C. REINDORP, 1939. Endomitosis in the nurse cells of the ovary of *Drosophila melanogaster*. *Chromosoma*, **1**: 276–283.

STALKER, H. D., 1954. Banded polytene chromosomes in the ovarian nurse cells of adult Diptera. *J. Heredity*, **45**: 259–264.